

collagen expression through ALK5 in these cells. Our findings suggest that hypoxic culture conditions for cartilage tissue engineering may benefit from an auto-regulatory cytokine signalling loop.

<http://dx.doi.org/10.1016/j.jot.2016.06.197>

#### TGF $\beta$ SUPERFAMILY-DEPENDENT AND OSMOLARITY-MEDIATED TYPE II COLLAGEN EXPRESSION BY CHONDROCYTES *IN VITRO*

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**Background:** *In vitro* expansion of human articular chondrocytes (HACs) is required for cell-based therapies to treat cartilage pathologies, because cartilage has a poor intrinsic repair capacity. During standard expansion culture (i.e., plasma osmolality, 280 mOsm/kg) chondrocytes inevitably lose their specific phenotype and de-differentiate. Unfortunately, this makes them inappropriate for autologous chondrocyte implantation, or related, chondral repair techniques. It has been shown that slightly elevated, but for articular chondrocytes physiological, osmolality (i.e., 380 mOsm/kg) increases type II collagen (COL2) expression *in vitro*. The underlying molecular mechanism is, however, currently unknown. The transforming growth factor beta (TGF $\beta$ ) superfamily members are accepted key regulators of chondrocyte differentiation and further well-known to stimulate COL2 synthesis in a variety of cell types, among which are chondrocytes. In this study, we aimed to elucidate the contribution of the TGF $\beta$  superfamily signalling as a putative molecular mechanism that potentially stimulates COL2 expression under physiological hyperosmolality *in vitro*.

**Subjects and Methods:** HACs(P1) from patients undergoing knee arthroscopy were cultured in cytokine-free medium of 280 or 380 mOsm/kg, under standard 2D *in vitro* conditions, with or without lentiviral TGF $\beta$ 2 RNA interference (RNAi, Sigma-Aldrich shRNA library). Expression of TGF $\beta$  isoforms, BMPs, and chondrocyte marker genes was evaluated by real-time qRT-PCR. TGF $\beta$ 2 protein secretion was evaluated using ELISA and bioactivity was determined using an established reporter cell line. Involvement of BMP signalling was further investigated by culturing HACs in the presence or absence of dorsomorphin (10  $\mu$ M), an established pharmacological inhibitor of these cytokines.

**Results:** Physiological osmolality increased TGF $\beta$ 2 and TGF $\beta$ 3 mRNA expression, TGF $\beta$ 2 protein secretion, as well as general TGF $\beta$  activity under 380 mOsm/kg.

Upon TGF $\beta$ 2 isoform-specific shRNA-mediated knockdown, COL2 mRNA expression was induced (Fig. 1). TGF $\beta$ 2 RNAi further induced expression of several BMPs to large divergent extents (Fig. 1, data not shown), which were further up-regulated under physiological osmolality (Fig. 1). Physiological osmolality-induced COL2 mRNA expression was, in contrast, secondarily suppressed by BMP-inhibitor dorsomorphin (data not shown).

**Discussion and Conclusion:** TGF $\beta$ 2 knockdown under 380 mOsm increases COL2 expression in human chondrocytes *in vitro*, most likely through feedback regulation of BMP signalling. BMP signalling itself is induced by osmolality and increases COL2 expression. Future studies will elucidate the BMP-mediated regulatory feedback loop upon TGF- $\beta$ 2 knockdown in more detail, as well as its influence on COL2 expression.

<http://dx.doi.org/10.1016/j.jot.2016.06.198>

#### EFFECTS OF PLATELET-RELEASED GROWTH FACTORS AND MONOAXIAL STRAIN ON TENOCYTES *IN VITRO*

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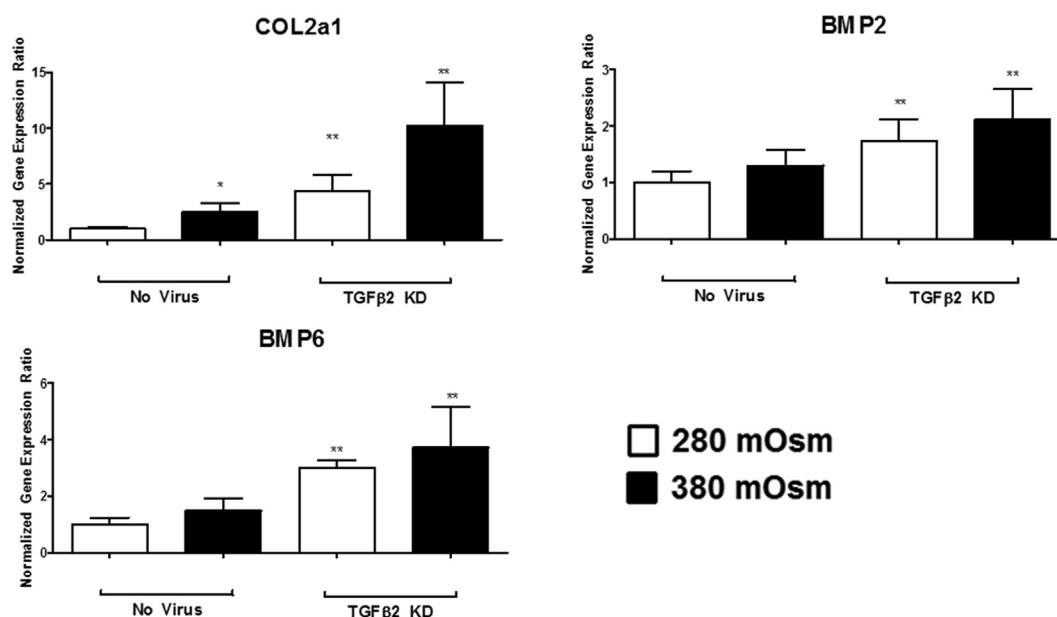
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**Background:** Ruptured tendons heal very slowly and complete recovery from injury is uncertain. Hypo-cellularity and poor nutrient supply through a lack of vascularity make tissue regeneration challenging. In addition, tenocytes tend to de-differentiate during routine *in vitro* culture, while tendon-derived progenitor cells (TDCs) can differentiate into mature tenocytes under a variety of physiological stimulations. As tendons naturally transmit loads between muscles and bones, they withstand substantial levels of strain *in situ*. Tensile strain is thus, an important environmental factor for tendon homeostasis from which regenerative *in vitro* approaches may benefit. However, current evidence is ambiguous and cyclic tensile strains of different frequencies and amplitudes appear to exert different effects, ranging from promoting tenogenic differentiation, to inducing non-tenocyte lineage differentiation. Platelet-rich plasma (PRP) is currently being widely clinically tested as a soft tissue-healing agent and may accelerate tendon repair. More specifically, platelet-released growth factors (PRGF) may promote TDCs differentiation and stabilise their phenotype *in vitro*. Here, we used a novel



**Figure 1** Relative normalised COL2, BMP2, BMP4 and BMP6 mRNA expression in 280 (white) and 380 (black) mOsm cultures and upon TGF- $\beta$  isoform-specific knockdown (KD). Expression ratios of 280 mOsm control condition without virus are set to 1. Differences from control condition are indicated by \*\* ( $p < 0.005$ ).